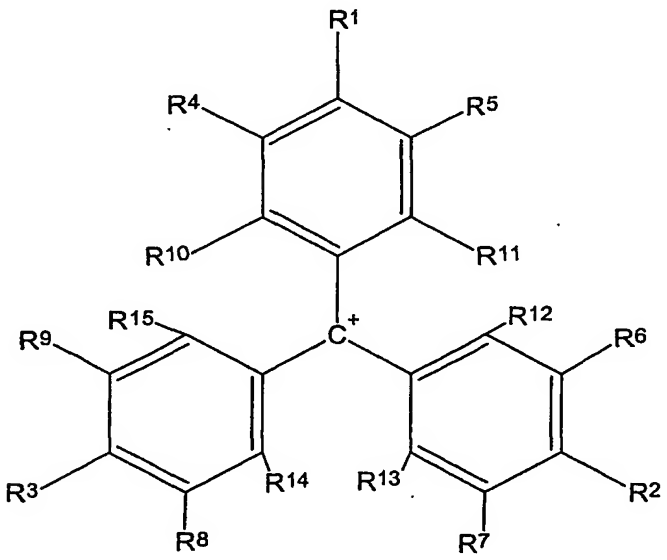
Claims

1. A reagent for use in detecting an analyte, comprising a fluorescent energy donor and an energy acceptor, the energy donor and the energy acceptor being such that when they are sufficiently close to one another energy is non-radiatively transferred from the energy donor following excitation thereof to the energy acceptor quenching fluorescence of the energy donor, wherein the energy acceptor is of the general formula:



wherein:

R^1, R^2 and R^3 are each independently H, electron donating substituents, or electron withdrawing substituents or R^3 is attached to a linker structure, provided that at least two of R^1, R^2 and R^3 are electron donating groups;

R^4, R^5, R^6, R^7, R^8 and R^9 are each independently H, halogen, alkyl, aryl, O-alkyl, S-alkyl and R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are each independently hydrogen, O-alkyl, S-alkyl, alkyl, or one or more pairs of groups R^1 and R^4 and/or R^1 and R^5 and/or R^2 and R^6 and/or R^2 and R^7 and/or R^3 and R^8 and/or R^3 and R^9 and/or R^4 and R^{10} and/or R^5 and R^{11} and/or R^6 and R^{12} and/or R^7 and R^{13} and/or R^8 and R^{14} and/or R^9 and R^{15} is a bridging group consisting of aryl, alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO_3^-, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-dialkyl, SO_3-alkyl, CN, secondary amine or tertiary amine, provided that not all of R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are hydrogen;

and wherein the distance between the energy donor and the energy acceptor of the reagent is capable of modulation by a suitable analyte to be detected.

2. A reagent as claimed in Claim 1, wherein the energy donor and energy acceptor are linked together by a covalent linkage.

3. A reagent as claimed in Claim 2, wherein the covalent linkage between the energy donor and energy acceptor is cleavable to increase the distance between the energy donor and the energy acceptor of the reagent.

4. A reagent as claimed in Claim 2, wherein the energy donor and energy acceptor are linked via a

polynucleotide sequence or a polynucleotide analogue sequence or a polypeptide sequence, the sequence having a conformation which is capable of modulation by a suitable analyte to be detected so as to modulate the distance between the energy donor and the energy acceptor of the reagent.

5. A reagent as claimed in Claim 1, wherein the energy donor and energy acceptor are linked together by non-covalent binding.

6. A reagent as claimed in Claim 5 wherein the non-covalent binding exists between an analyte binding agent linked to one of the energy donor and the energy acceptor and an analyte analogue linked to the other of the energy donor and the energy acceptor, the non-covalent binding being disruptable by a suitable analyte so as to increase the distance between the energy donor and the energy acceptor of the reagent.

7. A reagent as claimed in Claim 6, wherein the analyte binding agent is a lectin.

8. A reagent as claimed in Claim 6 or Claim 7, wherein the analyte analogue is a glucose analogue.

9. A reagent as claimed in Claim 8, wherein the analyte analogue is dextran.

10. A reagent as claimed in Claim 1, wherein the energy donor and the energy acceptor are not linked in the absence of analyte.

11. A reagent as claimed in any one of the preceding claims, wherein a linker structure is attached to the energy acceptor at R^3, or where a bridging group is present optionally the linker structure is attached to the energy acceptor at the bridging group.

12. A reagent as claimed in any one of the preceding claims, wherein the electron donating substituents are selected from amino, primary amine, secondary amine, O-alkyl, alkyl, S-alkyl, amide, ester, OH and SH.

13. A reagent as claimed in Claim 12, wherein one or more of R^1 to R^3 is dimethylamino, diethylamino or methylethylamino, optionally substituted with one or more of SO_3^-, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-dialkyl, SO_3-alkyl, CN, secondary amine or tertiary amine.

14. A reagent as claimed in any one of the preceding claims, wherein an electron withdrawing substituent is present, and the electron withdrawing substituent is selected from NO, NO_2, CN, COOH, ester, COO^-, amide, CHO, keto, SO-alkyl, SO_2-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-dialkyl, and SO_3-alkyl.

15. A reagent as claimed in any one of the preceding claims, wherein at least one of R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} is O-alkyl.

16. A reagent as claimed in any one of the preceding claims, wherein one or more pairs of groups R^4 and R^{10} and/or R^5 and R^{11} and/or R^6 and R^{12} and/or R^7 and R^{13} and/or R^8 and R^{14} and/or R^9 and R^{15} is a bridging group consisting of alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO_3^-, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-dialkyl, SO_3-alkyl, CN, secondary amine or tertiary amine.

17. A reagent as claimed in any one of Claims 1 to 14, wherein R^{10} to R^{15} are each O-methyl or O-ethyl.

18. A reagent as claimed in any one of the preceding claims, further comprising one or more counterions selected from halide, BF_4^-, PF_6^-, NO_3^-, carboxylate, ClO_4^-, Li^+, Na^+, K^+, Mg^{2+} and Zn^{2+}.

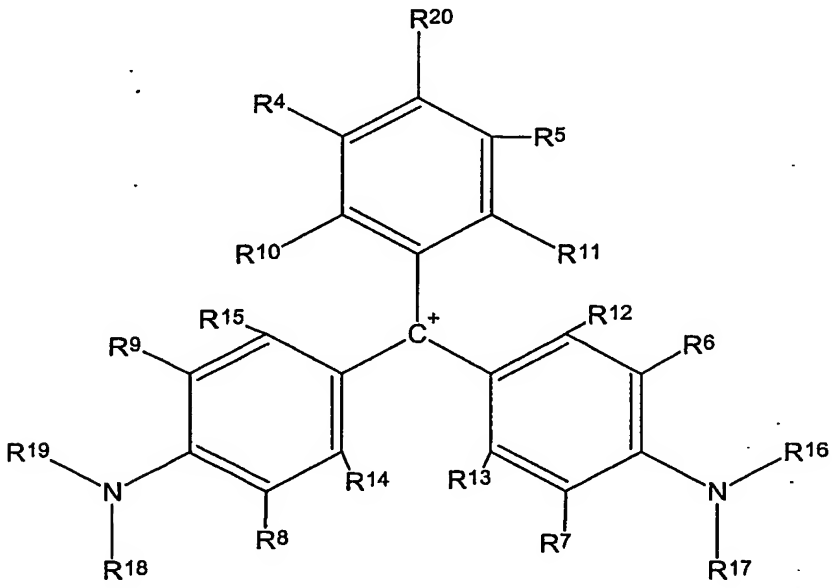
19. A reagent as claimed in any one of the preceding claims, wherein a linker structure is present, and is formed by reaction of a linker element selected from an active ester, an isothiocyanate, an acid chloride, an aldehyde, an azide, an α-halogenated ketone and an amine with a reaction partner.

20. A reagent as claimed in Claim 19, wherein the reaction partner is selected from a polysaccharide, a polynucleotide and a protein.

21. A reagent as claimed in Claim 19 or Claim 20, wherein the linker element is an active ester, and is selected from succinimidyl and pentafluorophenyl active esters.

22. A reagent as claimed in any one of the preceding claims, wherein the energy donor is Alexa Fluor-594™.

23. A dye compound having the general formula:



wherein:

R^4, R^5, R^6, R^7, R^8 and R^9 are each independently H, halogen, alkyl, aryl, O-alkyl or S-alkyl and R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are each independently hydrogen, O-

alkyl, S-alkyl, or alkyl, or one or more pairs of groups R^{20} and R^4 and/or R^{20} and R^5 and/or R^4 and R^{10} and/or R^5 and R^{11} and/or R^6 and R^{12} and/or R^7 and R^{13} and/or R^8 and R^{14} and/or R^9 and R^{15} is a bridging group consisting of aryl, alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO_3^-, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-dialkyl, SO_3-alkyl, CN, secondary amine or tertiary amine, provided that not all of R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are hydrogen;

R^{16}, R^{17}, R^{18} and R^{19} are each independently H, alkyl or aryl, or one or more of R^{16} and R^{17} or R^{18} and R^{19} is alkylene, optionally substituted with one or more of SO_3^-, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-dialkyl, SO_3-alkyl, CN, secondary amine or tertiary amine;

or one or more of pairs of groups R^6 and R^{16}, R^7 and R^{17}, R^8 and R^{18} and R^9 and R^{19} is alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO_3^-, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-dialkyl, SO_3-alkyl, CN, secondary amine or tertiary amine

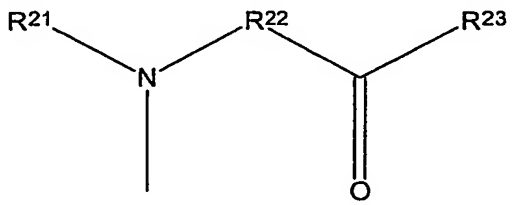
and

R^{20} is a linker element selected from an active ester, an isothiocyanate, an acid chloride, an α-halogenated ketone, an azide and an amine.

24. A dye compound as claimed in Claim 23, wherein at least one of R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} is alkyl.

25. A dye compound as claimed in Claim 24, wherein one or more pairs of groups R^4 and R^{10} and/or R^5 and R^{11} and/or R^6 and R^{12} and/or R^7 and R^{13} and/or R^8 and R^{14} and/or R^9 and R^{15} is a bridging group consisting of alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO_3, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-dialkyl, SO_3-alkyl, CN, secondary amine or tertiary amine.

26. A dye compound as claimed in any one of Claims 23 to 25, wherein R^{20} is a linker element having the structure:



R^{21} is H or alkyl or aryl optionally substituted with one or more of SO_3^-, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2N-dialkyl, CN, secondary amine or tertiary amine and R^{22} is alkylene, O-alkylene, S-alkylene or N-alkylene or R^{21} and R^{22} are part of a ring, optionally substituted with

one or more of SO_3^-, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-dialkyl, SO_3-alkyl, CN, secondary amine or tertiary amine; and

R^{23} is *o*-succinimidyl, *o*-pentafluorophenyl, Cl or α-halogenated alkyl.

27. A dye compound as claimed in any one of Claims 23 to 26, wherein R^{10} to R^{15} are each O-methyl or O-ethyl.

28. A dye compound as claimed in any one of Claims 23 to 27, further comprising one or more counterions selected from halide, BF_4^-, PF_6^-, NO_3^-, carboxylate, ClO_4^-, Li^+, Na^+, K^+, Mg^{2+} and Zn^{2+}.

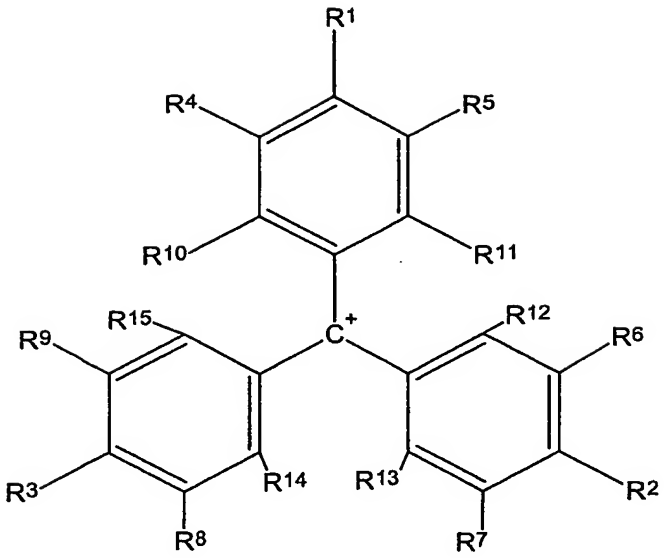
29. A method of detecting or measuring an analyte using a reagent as claimed in any one of Claims 1 to 22, comprising the steps of:

contacting the reagent with a sample;
illuminating the reagent and sample with light of wavelength within the absorption spectrum of the energy donor;
detecting non-radiative energy transfer between the energy donor and energy acceptor by measuring the fluorescence of the energy donor; and
associating the fluorescence measurements with presence or concentration of analyte.

30. A method as claimed in Claim 29, wherein the fluorescence of the energy donor is measured by measuring making intensity based or time resolved fluorescence measurements.

31. A method as claimed in Claim 29 or 30, wherein the analyte is measured by comparing sample fluorescence measurements with fluorescence measurements made using known concentrations of analyte.

32. A complex of an analyte and a reagent for detecting the analyte wherein the reagent comprises a fluorescent energy donor and an energy acceptor, the energy donor and the energy acceptor being such that when they are sufficiently close to one another energy is non-radiatively transferred from the energy donor following excitation thereof to the energy acceptor quenching fluorescence of the energy donor, wherein the energy acceptor is of the general formula:



wherein:

R^1, R^2 and R^3 are each independently H, electron donating substituents, or electron withdrawing substituents or R^3 is attached to a linker structure, provided that at least two of R^1, R^2 and R^3 are electron donating groups;

R^4, R^5, R^6, R^7, R^8 and R^9 are each independently H, halogen, alkyl, aryl, O-alkyl, S-alkyl and R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are each independently hydrogen, O-alkyl, S-alkyl, alkyl, or one or more pairs of groups R^1 and R^4 and/or R^1 and R^5 and/or R^2 and R^6 and/or R^2 and R^7 and/or R^3 and R^8 and/or R^3 and R^9 and/or R^4 and R^{10} and/or R^5 and R^{11} and/or R^6 and R^{12} and/or R^7 and R^{13} and/or R^8 and R^{14} and/or R^9 and R^{15} is a bridging group consisting of aryl, alkylene, O-alkylene, S-alkylene or N-alkylene optionally substituted with one or more of SO_3^-, PO_3^{2-}, OH, O-alkyl, SH, S-alkyl, COOH, COO^-, ester, amide, halogen, SO-alkyl, SO_2-alkyl, SO_2NH_2, SO_2NH-alkyl, SO_2N-

dialkyl, and SO_3-alkyl, CN, secondary amine or tertiary amine, provided that not all of R^{10}, R^{11}, R^{12}, R^{13}, R^{14} and R^{15} are hydrogen; and

wherein the presence of the analyte modulates the distance between the energy donor and the energy acceptor.